Proc. Arthropod. Embryol. Soc. Jpn. 43, 39–42 (2009)

ISSN 1341–1527

Reconstruction of Hexapod Basal Clades from Embryological Evidence*

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The phylogeny of Hexapoda at higher levels has been argued in various disciplines, but has not been settled (cf. Wheeler *et al*., 2001). Recently, doubt has been cast on the hexapod phylogenetic system, currently the most accepted, or the 'Entognatha-Ectognatha system' of Hennig (1969), especially regarding the status of Entognatha, by some approaches (*e. g*., Kukalová-Peck, 1987; Koch, 1997; Kraus, 1998), including our comparative embryological contribution (Ikeda and Machida, 1998, 2001; Machida *et al*., 2002). For phylogenetic argument, the comparative embryological approach is one of the most promising. In the past few decades, much embryological knowledge concerning hexapod basal clades has been accumulated. Reviewing these results, the hexapod basal clades are discussed and reconstructed from comparative embryological perspectives.

Monophyly of Diplura

The comparative morphology of ovariole types (cf. Biliński, 1994) and spermatozoa (cf. Jamieson, 1987) throws some doubt on the monophyletic status of Diplura; however, comparative embryological evidence strongly suggests its monophyly, based on the formation of the entognathy, the abdominal metamerism, and the formation of the primary dorsal organ.

Rhabdura (Campodeina) (Ikeda and Machida, 1998) and Dicellurata (Japygina) (Silvestri, 1933; Sekiya and Machida, 2009) share a peculiar form of entognathy, which is characterized by (1) mouth folds derived only from the mandibular and maxillary terga, (2) partitioning of the posterior part of the maxillary tergum into the admentum, and (3) rotation of the labial appendages involved. These features revealed in the dipluran entognathy are unique to Entognatha, and are too peculiar not to postulate their common acquisition by the two dipluran suborders Rhabdura and Dicellulata.

It is well known that both dipluran suborders possess only ten abdominal segments. The aberrant number of abdominal segments of Diplura has been often explained by the highly intensive reduction of the eleventh abdominal segment, which is represented only by cerci (cf. Matsuda, 1976); however, this may be misleading. Inspecting the development of the abdominal metamerism in Diplura, it is clear that ten as the number of abdominal segments is correct: no sign suggestive of an 'eleventh abdominal segment' exists and the cerci originally formed as appendages of the tenth abdominal segment (cf. Ikeda and Machida, 1998; Sekiya and Machida, 2009). Klass (2001) had come to the same conclusion based on a comparison of adult skeletomuscular morphology in Ectognatha.

In Rhabdura, the primary dorsal organ has been known to be formed by a concentration of serosal cells (Uzel, 1898; Ikeda and Machida, 2001), and this mode of formation of the primary dorsal organ, which is singular in Entognatha, has been regarded as one of the embryological characteristics of Diplura (cf. Ikeda and Machida, 2001). Recently, similar formation of the primary dorsal organ was revealed in Dicellurata (Sekiya and Machida, 2009).

Similarities in Rhabdura and Dicellurata in entognathy formation, abdominal metamerism and primary dorsal organ formation are good enough to postulate their common acquisition by these dipluran suborders, being regarded as embryological synapomorphies. The monophyly of Diplura is well supported.

Embryological features of Protura

Protura, although generally dealt with as one of the hexapod basal clades (*e. g*., Kristensen, 1975; Boudreaux, 1979), have been often regarded as representing a side branch remote from the main line of hexapod evolution (*e. g*., Sharov, 1966; Manton, 1977), and are thus important in reconstruction of the relationships of hexapod basal clades and elucidation of the origin of hexapods. Recently, evidence suggestive of a closer affinity between Myriapoda and Chelicerata (or some of

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* Contribution to the symposium "Reconstruction of Hexapod Phylogeny: An Overview from Comparative Reproductive Biology." The 43rd Annual Meeting of the Arthropodan Embryological Society of Japan, July 5, 2007 (Sugadaira, Japan).

each) and that between Hexapoda and Crustacea has emerged (*e. g.*, Aguinaldo *et al.*, 1997; Hwang *et al.*, 2001; Sakuma and Machida, 2002), and the monophyletic status of Atelocerata (=Myriapoda + Hexapoda) and Mandibulata (=Atelocerata + Crustacea) should be reexamined. In this background, embryological research on Protura is required more than ever. Our former total ignorance of proturan embryogenesis, in spite of many attempts, was due to the difficulty in rearing proturans. Recently, we succeeded in rearing proturans (Machida and Takahashi, 2003, 2004) and obtained a sufficient number of eggs, and we are now outlining their embryonic development. Our ongoing study has revealed some intriguing embryological features of Protura (Fukui and Machida, 2006).

The general features of embryogenesis in proturans are similar to those in the likewise entognathous collembolans (cf. Jura, 1972) and diplurans (cf. Ikeda and Machida, 1998, 2001) in that (1) the embryo is the long germ type, (2) simple blastokinesis involving only a minor change of posture occurs, (3) a primary dorsal organ forms, and (4) the embryo, together with the serosa, secretes a cuticular egg envelope or blastoderm cuticle. On the other hand, in proturan embryogenesis, the serosa is able to differentiate into the dorsal body wall – utterly different from the situation in the other entognathous (and hexapod) taxa, in which the serosa eventually degenerates without participating in the body wall formation. The embryonic membrane of Protura is represented only by the serosa. In this respect, Protura resemble Collembola (cf. Jura, 1972) but differ from Diplura in which a second embryonic membrane or amnion develops (cf. Ikeda and Machida, 1998, 2001).

The entognathy formation of Protura remains to be clarified, but our preliminary observations (Fukui and Machida, 2009) suggest that the three gnathal terga and intercalary element, which fuses with the clypeolabral anlage, participate in the formation of the mouth folds.

Status of Entognatha

Hennig's 'Entognatha-Ectognatha System' (1969) has been widely accepted, with little challenge. The taxon Ectognatha is indeed well established and supported by various disciplines, including our comparative embryological evidence, as mentioned below. However, the taxon Entognatha may be fragile, because it is supported by very few features, a main point of which is the entognathy. Recent comparative paleontological (Kukalová-Peck, 1987), comparative morphological (Koch, 1997), and our comparative embryological (Ikeda and Machida, 1998) approaches have provided notable evidence discounting the validity of entognathy, the most powerful autapomorphy of the group.

As mentioned above, the entognathy in Diplura is formed by a unique plan, and the entognathy in Collembola may have an utterly different plan. In Collembola, unlike in Diplura, the mouth folds are derived from the mandibular, maxillary and labial terga, and neither the formation of the admentum nor rotation of the labial appendages is involved in the formation of the entognathy (cf. Uemiya and Ando, 1987). The differences in entognathy formation in Collembola and Diplura may be too significant to postulate a common acquisition by these two orders. In other words, they may strongly suggest that the entognathy has been acquired in a convergent and parallel manner in the lineages of Collembola and Diplura. Concerning these two entognathous orders at least, the entognathy loses its validity as a synapomorphy (Ikeda and Machida, 1998). Entognathy formation in Protura, which is yet to be sufficiently clarified, is probably different from that in either Collembola or Diplura (Fukui and Machida, 2009).

Protura (Fukui and Machida, 2006), Collembola (cf. Jura, 1972), and Diplura (Uzel, 1898; Ikeda and Machida, 1998, 2001; Ikeda, 2001; Sekiya and Machida, 2009) have significant differences in embryological features other than the entognathy: (1) cleavage type, which in Protura and Collembola is holoblastic or total, and in Diplura is superficial; (2) the manner of endodermal and mesodermal segregation, which in Collembola is not so unusual, but in Diplura is absolutely unique (no data for Protura); (3) abdominal metamerism, which is unique in each order as hexapods, but in different ways, Protura having nine initial segments, increasing to 12 during the postembryonic stage, Collembola having six segments and Diplura having ten segments; and (4) the embryonic membrane, represented in Protura and Collembola only by the primary embryonic membrane or the serosa, whereas in Diplura, a second embryonic membrane or the amnion differentiates; the serosa in Protura retains the ability to differentiate into the body element as do those in more primitive arthropods such as Myriapoda or Crustacea, but those in Collembola and Diplura have lost such an ability during evolution, as well as in other hexapods or Ectognatha.

There exist substantial embryological differences among Protura, Collembola and Diplura. Furthermore, the entognathy has been regarded as the most reliable autapomorphy of Entognatha, but its homology has proved to be doubtful; hence, the monophyly of Entognatha is not always substantiated.

Reconstruction of hexapod basal clades, based on the evolutionary transition of embryonic membranes

Machida *et al.* (1994) and Machida and Ando (1998) demonstrated evolutionary changes in embryonic membranes and anagenetical transition of developmental potentials (involving dorsal closure and secretion of the cuticular egg envelope) in the embryo proper and embryonic membranes in Hexapoda. Incorporating new information on the embryonic membranes of Diplura (Ikeda and Machida, 2001), Zygentoma (Masumoto and Machida, 2006) and Protura (Fukui and Machida, 2006), their concept was revised by Machida *et al*. (2002) and Machida (2006), to be summarized as follows.

(1) The most primitive condition is when no definite functional specialization has taken place between the embryo and embryonic membrane, which is represented by the serosa, concerning dorsal closure and secretion of the cuticular egg envelope. Such a condition, shown in candidates of the hexapod sister group Myriapoda or Crustacea, is regarded as the most plesiomorphic within Hexapoda, and is represented only by Protura. (2) In advance of Protura, the embryonic membrane (serosa) ceases participation in definitive dorsal closure (*i. e*., differentiation into the body element). Consequently this task is performed exclusively by the embryo, and the function of dorsal closure by the embryonic membrane is restricted to provisional dorsal closure. (i) In Collembola, the embryonic membrane remains to be represented by the serosa, but (ii) in Diplura and Ectognatha, a second embryonic membrane is acquired, and provisional dorsal closure is temporally achieved by the serosa and amnion. (3) In Ectognatha, the embryo loses the ability to secrete the cuticular egg envelope, resulting in acquisition of the embryonic membrane fold (serosal or amnioserosal) for the sake of cuticular secretion beneath (ventrally to) the embryo. The most primitive condition is represented by Archaeognatha, in which the embryonic membrane fold is ephemeral in structure. (4) In Dicondylia, the embryonic membrane fold or the amnioserosal fold is well integrated in embryogenesis, and the 'amnioserosal fold – amniotic cavity system (ASF – AC system)' is established. The most primitive condition is represented by Zygentoma, in which the formation of the ASF – AC system is still flexible. (5) Finally, in Pterygota, there is a close link between the formation of the embryonic membrane fold and production of the amnion, and formation of the ASF – AC system is fixed.

Tentative conclusion and perspectives

Figure 1 is a tree, on which the critical steps concerning the evolutionary transition of the embryonic membranes mentioned in the previous section are mapped, each of which may be evaluated as the autapomorphy for each clade. Both taxa Dicondylia and Ectognatha are supported as well as suggesting a sister group relationship of Diplura and Ectognatha. On the other hand, not only are Entognatha dismissed, but Hexapoda and Ellipura are not positively supported either. As aforementioned, the embryonic membrane (serosa) of Protura retains a very primitive condition, comparable to that of lower arthropods or the Myriapoda or Crustacea, different from that of other hexapods or Collembola, Diplura and Ectognatha. In discussing the affinity of Protura, we should pay much more attention to more primitive arthropods such as Myriapoda or Crustacea.

For phylogenetical reconstruction of hexapod basal clades, the most important task is the accumulation of embryological information on Protura. Critical embryological reexaminations of Collembola are also desired, for which embryological knowledge should be reexamined and tested using the framework followed here. Dipluran Dicellurata likewise deserve comprehensive embryologi-

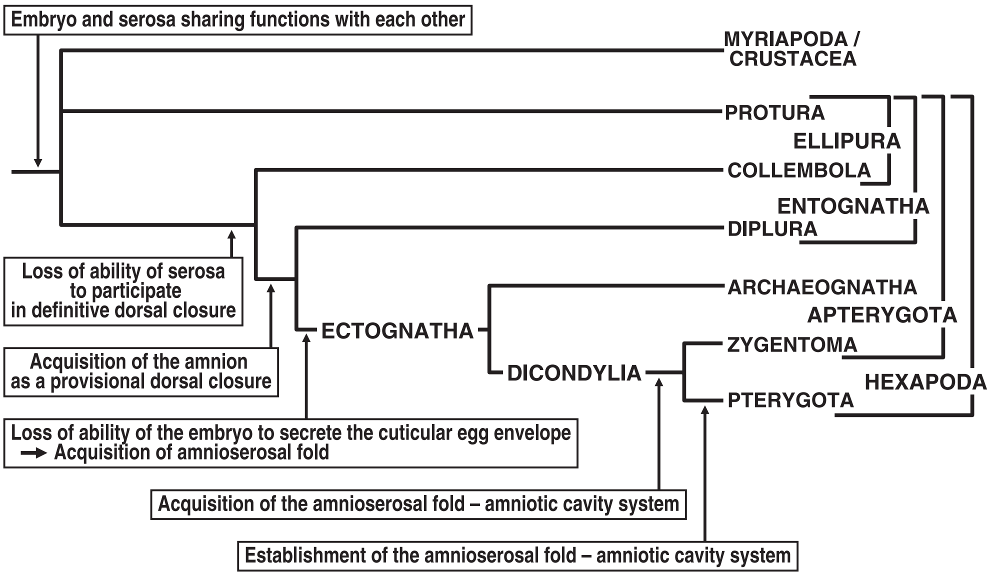

Fig. 1 Reconstruction of hexapod basal clades in the light of evolutionary changes of embryonic membranes and functional specialization in the embryo proper and embryonic membranes. See the text.

cal study, since we have little classical embryological knowledge on this group.

Acknowledgments: This research was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Scientific Research B: 17370030). This is contribution No. 211 from the Sugadaira Montane Research Center, University of Tsukuba.

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